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Narcotics and Rat Testicular Metabolism

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SUMMARY

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The effect of various narcotic analgesics on protein and nucleic acid synthesis in rat testicular cell suspensions and on testosterone production in rat testis was studied. Racemic methadone (*dl*-Me), *l*- and *d*-Me, *l*-α-acetyl-methadol (LAM), and *dl*-Me metabolites, 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline (EMDP) and 2-ethyl-1,5-dimethyl-3,3-diphenyl-pyrrolinium (EDDP), at 100 and 10 μM had a dose-related, significant effect on L-[1-^{14}C]leucine and [2-^{14}C]uridine incorporation into protein and RNA, respectively. LAM metabolites, methadol (MOL), acetylnormethadol (ANMOL) and normethadol (NMOL), were also inhibitory. Under the same conditions, morphine (Mo), heroin (He) and codeine (Co) had only minor effects on protein and RNA synthesis in comparison with *dl*-Me. The effect of narcotic analgesics on DNA synthesis was less than on RNA. The order of potency in inhibiting these biosynthetic process is: LAM ≈ *dl*-Me ≈ *l*-Me ≧ *d*-Me − EMDP > EDDP > MOL ≧ ANMOL ≧ NMOL ≫ He > Co > Mo. Furthermore at 1 mM *dl*-Me, *l*-Me and LAM caused about a 50% inhibition of testosterone (T) production and release from rat testes stimulated by hCG or dibutyryl-cAMP. In comparable experiments He or Mo were far less inhibitory. Further, the T production by dibutyryl-cAMP stimulated testicular Leydig cell preparations was also reduced by *dl*-Me and the ID_{50} was about 9 μM. The results suggest that some narcotic analgesics such as *dl*-Me, LAM and their metabolites may reduce testicular T synthesis by virtue of their direct inhibitory effects on RNA and/or protein synthesis. However, He and Mo may decrease T levels primarily through the hypothalamic-pituitary-gonadal axis. A combination of central and peripheral action of some narcotics cannot be excluded.

INTRODUCTION

Methadone, the synthetic narcotic analgesic, is widely used in "methadone maintenance programs" for the chronic treatment of morphine (Mo)[1] and heroin (He) dependent persons. The main goal of this treatment is to suppress drug hunger and prevent withdrawal symptoms. According to both clinical reports and animal experiments, however, there are many side effects

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[1] The abbreviations used are: Mo, morphine; He, heroin; *dl*-Me, *dl*-methadone; LAM, *l*-α-acetylmethadol; T, testosterone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; Co, codeine; EMDP, 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline hydrochloride; EDDP, 2-ethyl-1,5-dimethyl-3,3-diphenyl-pyrrolinium perchlorate; MOL, (−)-α-methadol hydrochloride; NMOL, (−)-α-normethadol perchlorate; ANMOL, (−)-α-N-acetyl-N-normethadol; TCA, trichloroacetic acid; ID_{50}, mean infective dose.

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from chronic use of any of the narcotic analgesics, but especially of *dl*-methadone (*dl*-Me) and its long-acting congener, *l*-α-acetylmethadol (LAM). The most prominent side effects are decreased sexual drive and performance (1–3), reduction of weight and function of testis (4) and secondary sex organs (2, 4), as well as decreased plasma levels of FSH, LH (1, 5) and testosterone (T) (2, 5, 6, 7).

The mechanism(s) underlying these side effects is not understood nor is it clear whether they are due to Me and LAM or to their metabolites. The occurrence of narcotic-related decreases in peripheral LH has been used as argument that alterations in testicular function and steroidogenesis may be due to a central action of narcotic analgesics via the hypothalamic-pituitary-gonadal axis (1, 5, 8, 9). A direct peripheral action of some narcotic analgesics upon the male gonads and related functions has, however, not been ruled out, although morphine in concentrations up to 1 mM did not affect the T production either by nonstimulated or hCG stimulated whole rat testis *in vitro* (5). Testicular T production has been shown to be dependent upon continuous synthesis of new RNA and protein in Leydig cells (10–12). In view of recent reports on the inhibition of nucleic acid and protein synthesis in testicular cells *in vitro* by *dl*-, *d*- and *l*-Me and LAM (9, 13), it seems possible that a direct inhibitory action of Me or LAM on testicular steroidogenesis contributes to or perhaps accounts for the reduction of T levels in male narcotic users. There are a few other recent reports indicating the possibility of direct peripheral effects of some narcotic analgesics on the target organs (2, 4, 6).

We here report the effects of LAM, Mo, He, codeine (Co) and some metabolites of Me and of LAM on nucleic acid and protein synthesis by rat testicular tissue, as well as the *in vitro* effects of Me, LAM, Mo, He and naloxone on testosterone production by rat testis and/or testicular Leydig cell preparations.

MATERIALS AND METHODS

Codeine phosphate, and *dl*-methadone hydrochloride were supplied by the local pharmacy. Samples of *d*- and *l*-methadone were generously provided by Dr. R. J. Hosley from the Lilly Research Laboratory, Indianapolis, Indiana, U. S. A. Morphine sulfate, heroin hydrochloride, 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline hydrochloride (EMDP), 2-ethyl-1,5-dimethyl-3,3-diphenyl-pyrrolinium perchlorate (EDDP), LAM, (−)-α-methadol hydrochloride (MOL), (−)-α-N-normethadol perchlorate (NMOL) and (−)-α-N-acetyl-N-normethadol (ANMOL) were supplied by the Department of Health and Welfare, Food and Drug Directorate, Ottawa, Canada. Naloxone was generously provided by Endo Laboratory. The testosterone [^{3}H] radioimmunoassay packs were purchased from Diagnostics Biochem. Canada, Inc., London, Ontario. Human chorionic gonadotropin from human pregnancy urine (hCG), collagenase from Cl.histolyticum Type I, and dibutyryl-cAMP were obtained from Sigma Chemical Company, St. Louis, Mo., U. S. A.

Effect of drugs on protein and nucleic acid synthesis. Testicular cell suspensions were always prepared from two testes of adult Wistar rats (250–300 g), stripped of the tunica and chopped into 0.3 mm segments with a McIllwain tissue chopper. The chopped tissue was suspended in 30 ml of Krebs-Ringer phosphate buffer (pH 7.4) with 10 mM glucose. After setting for 3 min, the cell suspension was separated from the sediment at the bottom by decantation. A second suspension and decantation was carried out using 15 ml of buffer. An aliquot of the combined cell suspensions was incubated for 60 min at 37° under O_2 in a final volume of 3 ml buffer (14). Metabolic activities were linear with time during such incubations. The drug was added in aqueous solution. The solution containing radioactive substrate was tipped into the main vessel from the side-arm of the Warburg flask (13).

After the incubation was terminated, the content of each Warburg flask was centrifuged, the sediment washed with 3 ml cold buffer and after centrifugation, the sediment was homogenized in 10% TCA. The TCA soluble fraction containing low molecular materials was separated from the precipitate by centrifugation. The precipitate

was then washed two times with cold 5% TCA and finally dissolved in Soluene 100 solubilizer (13, 14). Aliquots of the TCA soluble fraction as well as of the Soluene solutions were used for scintillation counting of radioactivity.

When [2-^{14}C]uridine was the substrate, various labeled uridine metabolites in the TCA soluble fraction were separated by chromatography on Whatman No. 1 paper in butanol:acetic acid:water (2:1:1 v/v/v) as solvent (13, 14). The areas corresponding to uridine and its metabolites were cut out and the radioactivity in each was counted in a liquid scintillation counter. The di- and tri-phosphonucleotides were not separated under this condition and thus were counted together (13, 14).

Total protein was estimated by colorimetry (15).

Effect on rat testicular steroidogenesis. Each testis obtained from Wistar rats (275–300 g) was weighed, decapsulated, washed in 0.9% NaCl and individually incubated in a vial containing 2.0 ml Krebs-Ringer bicarbonate buffer with 10 mM glucose, pH 7.4 at 37° for 30 min under O_2:CO_2 (95%:5%). The medium was syphoned off and the testis transferred to a new vial containing Krebs-Ringer phosphate buffer and additives in a final volume of 3.1 ml. The vials were flushed with 95% O_2:5% CO_2, capped and agitated at 37° in a water bath for 4 h (16). Nonstimulated incubations were in the absence of exogenous hCG or dibutyryl-cAMP. Control, stimulated incubations contained either 30 mIU hCG (11.4 ng) or 1.0 mM dibutyryl-cAMP. Experimental incubations were identical to control except for the presence of the narcotic added in the aqueous solution.

Each incubation medium containing one testis was then centrifuged and portions of the supernates, after dilution with 0.9% NaCl (1:25), were used for direct estimation of T by radioimmunoassay (16). All comparisons, control and experimental, were based on testes incubated and processed simultaneously for the T assay. Testes were randomly assigned to control and experimental groups except that the two testes from any given rat were always in separate groups.

Effect on Leydig cell steroidogenesis. Rat testicular Leydig cell preparations containing about 60% Leydig cells were prepared by the method of Janszen et al. (17). Decapsulated testes from adult male Wistar rats (250–300 g) were incubated in 7 ml Krebs-Ringer bicarbonate buffer with 10 mM glucose and with collagenase (1 mg/ml buffer) under O_2:CO_2 (95%:5%) for 25–30 min at 37° in plastic incubation vessels. After separation of the interstitial cells from the tubular mass by adding 15 ml 0.9% NaCl, the cell suspension was centrifuged through a 13% Ficol—0.4% albumin V buffer solution and finally through a 6% dextran in 0.9% NaCl solution (17). The sedimented cells were resuspended in 10 ml/testis of Krebs-Ringer phosphate buffer with 10 mM glucose, pH 7.4. Incubations of 1.9 ml of this cell suspension with 1.0 mM dibutyryl-cAMP and with or without *dl*-Me were carried out in a total volume of 2.1 ml buffer in scintillation vials under O_2 for 3 h at 37° in a Dubnoff metabolic shaker. All incubations were performed in triplicate. After incubation, aliquots of the incubation medium were used for direct radioimmunoassay of testosterone (16) produced by Leydig cells.

Statistical analyses were carried out using the Student's *t*-test.

RESULTS

Protein and nucleic acid metabolism in testicular cell suspensions. As previously reported [12], protein synthesis (as measured by the incorporation of radioactivity from L-[1-^{14}C]leucine into the protein fraction) as well as the released $^{14}CO_2$ was inhibited in a dose-related fashion by *dl*-Me in rat testicular cells. However, neither oxygen uptake nor the radioactivity in the cellular acid soluble fraction was affected (Table 1). Judged by their effects on the incorporation of radioactivity into protein, only EMDP of the other materials tested was as active an inhibitor as *dl*-Me. The others, less active compounds, fell in the rank order of activity: LAM > EDDP > ANMOL > MOL = NMOL > He > Mo > Co (Table 1). It is noteworthy that He, Mo and Co had significant effects only at concentrations much higher than those used

TABLE 1

Effect of narcotic analgesics on L-[1-^{14}C]leucine metabolism and incorporation in rat testicular cell suspension

Testicular cells were incubated in 3 ml Krebs-Ringer phosphate buffer with 10 mM glucose, under O_2, at 37° for 60 min, with 0.33 μCi of L-[1-^{14}C]leucine (3.3 μCi/μmol) as labeled substrate. Values are expressed as percentage (mean ± S.E.) of dpm of control incubations. Control values in dpm/mg protein were: $^{14}CO_2$ 2621 ± 94; soluble fraction 2443 ± 94; protein 858 ± 58; and μl O_2/mg protein 4.7 ± 0.2; $n = 20$.

Additives	Concentration	% of control			
		O_2	$^{14}CO_2$	Soluble Fraction	Protein
	(mM)		*(dpm/mg protein)*		
dl-Me	0.01	90 ± 5	85 ± 1*	91 ± 3	69 ± 9*
	0.05	90 ± 10	79 ± 5*	92 ± 2	46 ± 11*
	0.10	100 ± 10	70 ± 3*	112 ± 4	30 ± 3*
EMDP	0.01	95 ± 2	91 ± 1	89 ± 2	74 ± 2*
	0.05	89 ± 8	89 ± 3	91 ± 3	53 ± 2*
	0.1	72 ± 10*	71 ± 3*	71 ± 1*	27 ± 1*
EDDP	0.05	92 ± 7	91 ± 5	107 ± 2	83 ± 1*
	0.1	95 ± 6	80 ± 5*	109 ± 2	63 ± 1*
LAM	0.01	106 ± 6	94 ± 2	103 ± 2	95 ± 2
	0.05	110 ± 11	96 ± 1	113 ± 2	81 ± 2*
	0.1	106 ± 3	85 ± 1	107 ± 3	55 ± 4*
MOL	0.1	110 ± 6	100 ± 1	123 ± 2*	82 ± 1*
NMOL	0.1	114 ± 6	95 ± 2	107 ± 3	82 ± 1*
ANMOL	0.1	82 ± 9	91 ± 1	120 ± 1*	75 ± 3*
Mo	0.1	89 ± 3	84 ± 5	95 ± 3	92 ± 2
	0.5	92 ± 3	84 ± 3	90 ± 5	82 ± 4
	1.0	82 ± 3	86 ± 3	98 ± 4	71 ± 4*
Co	0.1	97 ± 2	102 ± 1	108 ± 2	104 ± 3
	0.5	94 ± 3	97 ± 6	136 ± 2*	87 ± 4
	1.0	97 ± 1	93 ± 4	157 ± 5*	70 ± 3*
He	0.1	100 ± 4	93 ± 1	107 ± 2	84 ± 5
	0.5	100 ± 8	88 ± 2	133 ± 4*	73 ± 3*
	1.0	100 ± 4	84 ± 4	157 ± 4*	61 ± 2*

* $p < 0.02$.

with Me, LAM and their metabolites. Some apparent differences from the effects of *dl*-Me were noted. Thus, EMDP at 0.1 mM significantly reduced both oxygen uptake and the amount of label in the soluble fraction while Co, He, MOL and ANMOL seemed to cause an increase in the label in the supernatant obtained by homogenization of the tissue pellet in TCA soluble fraction, (Table 1).

As previously reported (13) *dl*-Me also caused a marked and dose-related decrease in RNA synthesis, as measured by the incorporation of radioactivity from [2-^{14}C]uridine (Table 2). This occurred along with decreases in the amount of label in the phosphorylated derivatives, UMP, UDP and UTP. Similarly, the Me metabolites EMDP and EDDP, LAM and its metabolites MOL, ANMOL and NMOL, as well as

TABLE 2

Effect of narcotic analgesics on [2-^{14}C]uridine metabolism and incorporation in rat testicular cell suspension

Incubation done as described in TABLE 1 except that 0.33 μCi of [2-^{14}C]uridine (20 μCi/μmol) was used as labeled substrate. Control values in dpm/mg protein were: Soluble fraction 878 ± 48; RNA 143 ± 8; n = 30.

Additives	Concentration	% of control					
		Soluble Fraction	RNA	U	UR	UMP	UDP UTP
	mM	dpm/mg protein					
dl-Me	0.01	88 ± 2	74 ± 3*	96 ± 5	94 ± 2	73 ± 2*	67 ± 6*
	0.05	94 ± 4	66 ± 7*	104 ± 2	105 ± 2	67 ± 2*	63 ± 8*
	0.10	88 ± 4	39 ± 4*	99 ± 1	109 ± 2	52 ± 1*	35 ± 3*
EMDP	0.05	75 ± 1*	66 ± 1*	82 ± 1*	87 ± 1	53 ± 2*	47 ± 1*
	0.10	62 ± 2*	46 ± 2*	74 ± 3*	74 ± 1*	38 ± 1*	31 ± 1*
EDDP	0.05	85 ± 1	63 ± 2*	91 ± 1	90 ± 2	66 ± 1*	62 ± 2*
	0.10	84 ± 2	53 ± 1*	91 ± 2	94 ± 4	61 ± 3*	53 ± 2*
LAM	0.01	79 ± 3*	67 ± 3*	83 ± 4	90 ± 5	74 ± 7*	56 ± 5*
	0.05	79 ± 6*	48 ± 2*	88 ± 7	98 ± 8	53 ± 5*	39 ± 2*
MOL	0.05	90 ± 2	68 ± 1*	109 ± 2	95 ± 4	78 ± 2*	65 ± 1*
	0.1	87 ± 2	56 ± 3*	115 ± 2	97 ± 2	65 ± 4*	46 ± 3*
NMOL	0.1	86 ± 2	75 ± 3*	92 ± 1	95 ± 3	75 ± 6*	71 ± 3*
ANMOL	0.1	84 ± 2	59 ± 1*	98 ± 3	96 ± 2	70 ± 2*	54 ± 3*
Mo	0.1	100 ± 1	94 ± 2	102 ± 2	100 ± 1	101 ± 1	99 ± 6
	0.5	96 ± 2	91 ± 4	102 ± 2	99 ± 1	97 ± 3	87 ± 6
	1.0	96 ± 2	76 ± 8*	101 ± 4	107 ± 4	93 ± 5	75 ± 7*
Co	0.1	103 ± 4	93 ± 1	104 ± 4	107 ± 4	102 ± 1	94 ± 5
	0.5	101 ± 3	73 ± 4*	102 ± 2	114 ± 1	88 ± 7	79 ± 6*
	1.0	100 ± 2	65 ± 4*	100 ± 1	118 ± 2*	74 ± 5*	76 ± 2*
He	0.1	93 ± 9	83 ± 9	95 ± 5	99 ± 6	84 ± 9	81 ± 14
	0.5	102 ± 5	69 ± 3*	103 ± 5	111 ± 2	93 ± 5	88 ± 8
	1.0	102 ± 5	66 ± 4*	97 ± 4	127 ± 2*	86 ± 4	72 ± 7*

* $p < 0.02$.

He, Co and Mo, significantly reduced the incorporation of [^{14}C]uridine into RNA (Table 2), with a rank order of effectiveness very similar to that observed for protein synthesis. The relatively small effect of He, Co and Mo is again noteworthy. EMDP was again somewhat unique in that it appeared to reduce the amount of label in uridine and uracil as well as in the phosphorylated derivatives. EMDP and LAM both reduced the label in the total soluble fraction.

A few experiments on the incorporation of label from [$^{14}CH_3$]thymidine into DNA are summarized in Table 3. Even at a concentration of 0.05 mM *dl*-Me had a significant effect. The interference of He with DNA synthesis under these conditions was significant only at 1.0 mM and Mo had only a slight effect even at this concentration (Table 3).

In order to gain some insight as to whether these effects on protein and nucleic acid synthesis might be secondary to inhibition of energy metabolism, some comparable studies were done in the absence of

TABLE 3

Effect of dl-methadone, morphine and heroin on [$^{14}CH_3$]thymidine incorporation in rat testicular cell suspension

Incubation done as described in TABLE 1 except that 1 μCi of [$^{14}CH_3$]thymidine (59 μCi/μmol) was the labeled substrate. Control values in dpm/mg protein were: soluble fraction 2240 ± 78; DNA 46 ± 3; $n = 9$.

Additives	Concentration	% of control	
		Soluble Fraction	DNA
	mM	*dpm/mg protein*	
dl-Me	0.05	96 ± 1	72 ± 6*
	0.1	91 ± 1	62 ± 8*
Mo	1.0	101 ± 4	86 ± 8
He	1.0	102 ± 5	66 ± 4*

* $p < 0.02$.

exogenous glucose (Table 4). The respiration and the incorporation of radioactivity from L-[1-^{14}C]leucine into the soluble and protein fractions in the control incubations were all much less (50%, 76% and 39%, respectively) than found in the presence of exogenous glucose. The inhibitory effects of Me, LAM, Mo, Co and He on the metabolism and incorporation of [^{14}C]leucine by the testicular cell suspensions (Table 4A) were, however, closely comparable to the effects found in the presence of added glucose (Table 1), although in this case LAM seemed slightly more effective than *dl*-Me.

With [2-^{14}C]uridine as substrate in control incubations in the absence of glucose, the incorporation of label into RNA was only one-third of that seen in the presence of glucose, although the amount of radioactivity in the acid soluble fraction of testicular cells was comparable under the two conditions. The degree of inhibition by narcotics of RNA synthesis, as measured by incorporated radioactivity, was also comparable to that in the presence of exogenous glucose (Tables 4B and 2).

Neither *dl*-Me (up to 0.1 mM) nor Mo (up to 1.0 mM) had any significant effect on cellular ATP levels in testicular cell suspensions incubated in Krebs-Ringer phosphate buffer in the presence of glucose.

Stimulated and basal T production in rat testis in vitro. The effects of various narcotics upon *in vitro* T production in hCG and dibutyryl-cAMP stimulated rat testis are shown in Table 5. Exposure of stimulated testis to *dl*-Me or He caused dose-related decreases in testosterone production with *dl*-Me being much more potent than He. Mo seemed to be even less active than He. LAM and the separate isomers of Me at a concentration of 1 mM produce depletions in the amount of testosterone accumulated in the medium, which are similar to those produced by an equivalent dose of *dl*-Me. The opiate antagonist, naloxone, at 0.1 mM had no significant effect by itself and did not antagonize the inhibitory actions of *dl*-Me, Mo and He (Table 5).

When the effects of *dl*-Me and LAM on nonstimulated (without exogenous hCG or dibutyryl-cAMP) T production by the whole rat testis *in vitro* was examined, neither drug had a significant effect on basal androgen production even at 2.0 mM concentration.

Testosterone production in rat testicular Leydig cells in vitro. The exposure of dibutyryl-cAMP stimulated Leydig cell suspensions to *dl*-Me resulted in a concentration-dependent reduction of T present in the incubation medium (Fig. 1). The presence of 5 μM *dl*-Me (1.55 μg *dl*-Me/ml) caused a significant 40% inhibition of T production and the maximum 80% inhibition occurred at 50 μM concentration. The ID_{50} was about 9 μM, corresponding to about 2.8 μg *dl*-Me/ml (Fig. 1).

DISCUSSION

The present results demonstrate the direct interference of various narcotic analgesics, e.g., Me, LAM and some of their metabolites, with protein and nucleic acid synthesis in rat testicular cells. These drugs also markedly impaired the steroidogenic response induced by hCG and dibutyryl-cAMP in both whole rat testis and preparations of Leydig cells *in vitro*. Testicular cell suspensions presumably contain cells at various stages of spermatogenesis as well as interstitial cells. Protein synthesis has been shown to occur in all types of testicular cells except in mature spermatozoa, while DNA and RNA biosynthesis occur in

TABLE 4

Effect of narcotic analgesics on L-[1-^{14}C]leucine (A) and [2-^{14}C] uridine (B) metabolism and incorporation in rat testicular cell suspension

Incubation of rat testicular cells done as described in TABLE 1, except that no glucose was added to the incubation medium. The labeled substrate was 0.33 μCi of L-[1-^{14}C]leucine (A) or 0.33 μCi of [2-^{14}C]uridine (B). Control values in dpm/mg protein were: (A) $^{14}CO_2$ 1320 ± 63; soluble fraction 1868 ± 80; protein 333 ± 29. (B) Soluble fraction 823 ± 40; RNA 56 ± 3; and μl O_2/mg protein 2.9 ± 0.07; $n = 21$

Addition	Concentration	% of control		
		$^{14}CO_2$	Soluble Fraction	Protein
	mM		*dpm/mg protein*	
(A) L-[1-^{14}C]leucine:				
d- & *l*-Me	0.1	80 ± 1*	126 ± 1*	42 ± 2*
d-Me	0.1	88 ± 4	123 ± 1*	46 ± 1*
l-Me	0.1	73 ± 2*	115 ± 1*	40 ± 1*
LAM	0.1	79 ± 2*	103 ± 2	34 ± 2*
Mo	1.0	100 ± 4	105 ± 4	78 ± 4*
Co	1.0	100 ± 4	158 ± 5*	73 ± 5*
He	1.0	106 ± 2	162 ± 3*	79 ± 2*
				RNA
(B) [2-^{14}C]uridine:				
d- & *l*-Me	0.1		95 ± 2	42 ± 2*
l-Me	0.1		94 ± 1	44 ± 2*
d-Me	0.1		95 ± 2	49 ± 2*
LAM	0.1		89 ± 1	59 ± 5*
Mo	1.0		88 ± 5	61 ± 2*
Co	1.0		96 ± 3	66 ± 2*
He	1.0		107 ± 6	73 ± 7*

* $p < 0.02$.

the spermatogonia and elongated spermatids, respectively (18).

In the present experiments, significant inhibition of testicular macromolecular biosynthetic processes was brought about by a dose of *dl*-Me which is of the same order of magnitude as the Me concentrations found in saliva and gastric juice of former heroin addicts, following a single dose of Me (19). Concentrations of Me and its metabolites found at postmortem examination in human blood (some of which may have died of drug overdose) were of the same order of magnitude and even higher levels have been reported in bile, urine and some tissues (20).

Me may accumulate in various tissues including sexual organs (21). After one i.m. administration of *d*-Me to rabbits (about 10 mg/kg) the semen/blood concentration ratio was about 7. The concentrations of *d*-Me in the testis, prostate and seminal vesicles at 130 min after the drug administration were 4.3, 4.6 and 3.5 μg/g tissue, respectively, and were about 10 times higher than concentrations in blood (0.42 μg/ml) (21). In Me (20–80 mg) maintenance subjects the ratio of semen/blood Me concentrations ranged from 0.8–4.7 [22]. As far as we know, there are no data available on the possible accumulation of He or Mo in the sexual organs of humans. It may therefore be possible that drug concentrations high enough to have a direct effect on testicular cell biosynthetic processes are attained *in vivo*, particularly after repeated doses.

The present results also demonstrate the importance of the possible toxic effects of narcotic metabolites. In particular, the pharmacologically inactive metabolites of Me, EMDP and EDDP (23), were strong inhibitors of protein and nucleic acid syn-

TABLE 5

Effect of narcotic analgesics upon testosterone production by rat testis in vitro *in the presence of hCG or dibutyryl-cAMP*

Additives	Concentration	% of control testosterone[a]	
		hCG-stimulated	dibutyryl-cAMP-stimulated
	mM		
dl-Me	0.5	80 ± 1*	—
	1.0	57 ± 6*	62 ± 5*
	5.0	14.5 ± 1*	—
l-Me	1.0	—	55 ± 5*
d-Me	1.0	—	69.5 ± 5*
LAM	1.0	—	52 ± 8*
He	1.0	92.5 ± 5	—
	2.5	—	75 ± 8*
	5.0	51 ± 6*	—
Mo	2.5	—	82 ± 11
	5.0	61 ± 4*	—
Naloxone	0.1	88 ± 7	108 ± 7
He + Naloxone	5.0 ± 0.1	46 ± 3*	—
Mo + Naloxone	5.0 ± 0.1	61 ± 5*	—
	2.5 ± 0.1	—	106 ± 12
dl-Me + Naloxone	1.0 ± 0.1	—	65 ± 7*

[a] Control values of testosterone (in ng/g wet weight of testis) were 248 ± 11 (n = 7) (mean ± S.E.) in presence of hCG, and 312 ± 21 (n = 8) in presence of dibutyryl-cAMP. A total of 90 testes were used in these experiments. n = 3 or 4 for each experimental condition.

* $p < 0.01$ as compared with controls.

thesis (Tables 1 and 2) and T synthesis (unpublished data). The pharmacologically active major metabolites of LAM, MOL, NMOL and ANMOL (24, 25) were much less inhibitory under these conditions but still had significant effects. It seems therefore quite possible that these as well as other metabolites of Me or LAM might play important roles in some of the longer term, relatively subtle effects of narcotic usage in man [1–6], especially during pregnancy and on the developing fetus (26–28). This probability is strengthened when one realizes that Me, LAM and/or their metabolites have a slow clearance from the body (19, 24), easily cross the placenta, enter fetal circulation and accumulate in the fetal brain in a concentration several fold greater than in maternal brain (26).

The exact mechanism(s) by which narcotic analgesics affect cellular metabolism is not yet known. The results with [1-^{14}C] leucine (Tables 1 and 4A) suggest that lack of intracellular labeled substrate is not responsible for the inhibition of protein synthesis. With He and Co (Tables 1 and 4A) there is even a highly significant increase of label in the soluble fraction. Similarly, with [^{14}C]uridine as labeled substrate, the radioactivity in the soluble fraction was not affected, except with EMDP and LAM (Tables 2 and 4B). The oxygen consumption also was not affected by the narcotics (Table 1). These results, however, demonstrate an interference of the drugs with labeled RNA synthesis which correlates with decreased synthesis of labeled nucleotides and with the effects on protein synthesis. Thus, the impaired protein synthesis may be secondary, at least in part, to the decreased RNA synthesis which seems to involve inhibition of some phosphorylations. ATP levels under these experimental conditions were practically unaffected.

The great sensitivity of isolated whole rat testis (5, 16, 29), as well as Leydig cells (10–12), in their steroidogenic response to various stimulants (e.g., LH, hCG, dibutyryl-cAMP) is well documented. Cell suspensions have certain advantages in comparison with intact tissue incubations, e.g., there is a constant free access of the chemical to the cells. Access of the drug to the cells *in vivo*, especially on chronic administration, may be considerably greater than that achieved in brief *in vitro* experiments with intact tissue. It is thus of interest that responsiveness of Leydig cell preparations to the effect of *dl*-Me on T production (Fig. 1) is about 100 times higher than that shown by the whole rat testis (Table 5).

The inhibitory effects of *dl*-Me on T production in hCG stimulated, as opposed to nonstimulated whole rat testis (Table 5),

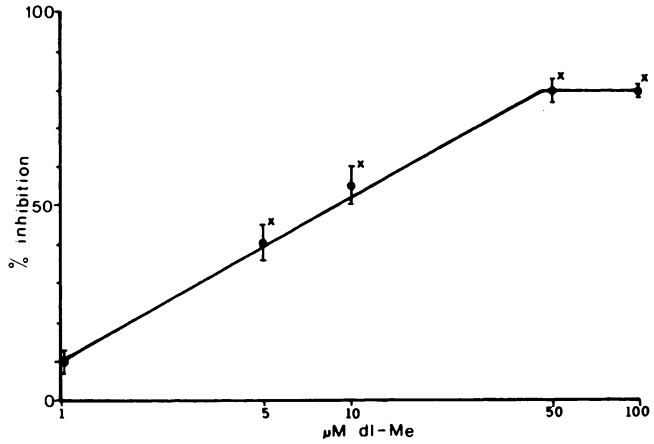

FIG. 1. *The effect of dl-Me on Leydig cells suspensions*

Suspensions prepared by collagenase digestion of decapsulated adult rat testes and purification through Ficol and dextran solution, were incubated for 3 h as described in METHODS. Control values of T (in ng/incubation) were: nonstimulated 1.75 ± 0.3; dibutyryl-cAMP stimulated 10.64 ± 0.04; $n = 3$. * $p < 0.01$ as compared with controls.

are similar to the experiments recently reported on human chopped testicular tissue (30) in which 0.5 mM *dl*-Me inhibited the hCG stimulated T formation from [^{3}H]cholesterol but did not affect the nonstimulated steroidogenesis (30). The precise mechanism of these stimulations is unknown. It has been shown, however, that T synthesis is related to constant RNA and protein synthesis in Leydig cells (10–12). Known inhibitors of protein and nucleic acid synthesis such as cycloheximide, puromycin or chloramphenicol reduced the stimulated T production in testicular preparations but did not affect basal synthesis of the androgen (10, 11). Similarly, Me or LAM, which inhibit RNA and protein synthesis in testicular cells (Tables 1, 2 and 4), had significant effects only on stimulated T production in the whole testis *in vitro* (Table 5; Fig. 1). Although the mechanism of stimulation of T production by dibutyryl-cAMP may differ from the mechanism of gonadotropin stimulation, the inhibition by analgesics was similar in the two systems (Table 5).

The previously reported (13) as well as the present biochemical effects (Tables 1–5) of these narcotic analgesics are different from their analgesic effects in that they are not stereospecific and are not antagonized by naloxone. The order of potency in inhibiting testicular macromolecular biosynthetic processes *in vitro*, was found to be: LAM ≈ *dl*-Me ≈ *l*-Me ≧ *d*-Me = EMDP > EDDP > MOL ≧ ANMOL ≧ NMOL ≫ He > Co > Mo. A very similar order was found for the inhibition of T production (Table 5), suggesting that these two effects may be related (11). This order of effectiveness is clearly different from that for analgesic action. In male participants on a methadone maintenance program the function of secondary sex-organs, as well as T levels, were found to be more suppressed than in heroin users or controls (2). However, heroin and methadone together lowered T plasma levels more than either drug alone [6]. These clinical findings and our present results are complementary.

The relative importance of central versus peripheral effects to narcotic-induced impairment of testicular steroidogenesis in addicts and the exact mechanism of this inhibition is unknown. If the inhibition of steroidogenesis in hCG stimulated testis by analgesics were related to changes in cellular cAMP production, one would expect less inhibition in the presence of exogenous dibutyryl-cAMP. This is not the case, however, suggesting that the inhibition of stimulated steroidogenes occurs at a step later than cellular cAMP formation. Furthermore, the effects of the narcotic analgesics

were not antagonized by naloxone (Table 5). The very weak effects of even extremely high concentrations of Mo and He on testicular biosynthetic processes (Tables 1–5) suggest that their *in vivo* actions on plasma T levels may be primarily central via the hypothalamic-pituitary-gonadal axis (5, 29). These data are, however, consistent with the interpretation (9, 13) that Me and LAM may depress T production through direct actions on the testis, which are mediated by their inhibiting effects on RNA and protein synthesis. The possibility of combined peripheral and central actions by some of the narcotic analgesics and their metabolites has also to be taken into consideration. Further *in vivo* experiments may provide more evidence for one or both mechanisms of action of Me and its congeners.

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